PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/19, C07K 14/52, 16/24, 19/00, A61K 38/19, C12Q 1/68, A61K 39/395

A1

(11) International Publication Number: WO 98/55621

(43) International Publication Date: 10 December 1998 (10.12.98)

(21) International Application Number: PCT/US98/11294

(22) International Filing Date: 3 June 1998 (03.06.98)

(30) Priority Data:
60/048,776 6 June 1997 (06.06.97) US
60/066,387 21 November 1997 (21.11.97) US

(71) Applicant *(for all designated States except US)*: REGENERON PHARMACEUTICALS, INC. [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591–6707 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MASIAKOWSKI, Piotr [PL/US]; Halter Lane, Box 44, Pleasant Valley, NY 12569 (US). VALENZUELA, David [CL/US]; 216 Grange Street, Franklin Square, NY 11010 (US).

(74) Agents: COBERT, Robert, J.; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591–6707 (US) et al.

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NTN–2 MEMBER OF TNF LIGAND FAMILY

(57) Abstract

HUMAN NTN–2 polypeptides and related nucleic acids are provided. Included are HUMAN NTN–2 polypeptides comprising a HUMAN NTN–2 domain having specific HUMAN NTN–2 activity. The polypeptides may be produced recombinantly from transformed host cells with the subject nucleic acids. Also provided are specific binding agents and methods of making and using the subject compositions.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

NTN-2 MEMBER OF TNF LIGAND FAMILY

All publications, patents and patent applications cited in this specification are hereby incorporated by reference as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

INTRODUCTION

Field of the Invention

The field of this invention is polypeptide molecules which regulate cell function, nucleic acid sequences encoding the polypeptides, and methods of using the nucleic acid sequences and the polypeptides.

Background

Tumor necrosis factor-alpha (TNF-alpha) is a cytokine primarily produced by activated macrophages. TNF-alpha stimulates T-cell and B-cell proliferation and induces expression of adhesion molecules on endothelial cells. This cytokine also plays an important role in host defense to infection.

TNF-alpha activities are mediated through two distinct receptors, TNFR-p55 and TNFR-p75. These two receptors also mediate activities triggered by soluble lymphotoxin-alpha (LT-alpha) secreted mainly by activated lymphocytes. Specific stimulation of TNFR-p55 induces TNF activities such as in vitro tumor cell cytotoxicity, expression of adhesion molecules on endothelial cells and keratinocytes, activation of sphingomyelinase with concomitant increases of ceramide, activation of NF-kappaB and induction of manganese superoxide dismutase mRNA. Specific stimulation of TNFR-

p75 results in proliferative response of mouse and human thymocytes and cytoxic T cells, fibroblasts and natural killer cells and in GM-CSF secretion in PC60 cells.

TNF, especially in combination with gamma.-interferon (IFN-.gamma.), has the ability to selectively kill or inhibit malignant cell lines that is unmatched by any other combination of cytokines. Clinical trials in cancer patients with TNF-.alpha. antitumor therapy have been disappointing, however, because the toxic side effects of TNF have prevented obtaining effective dose levels in man. These toxic side effects have been attributed to TNF binding to the TNFR-p75 receptor while the cytotoxic activity on malignant cells has been attributed to binding of TNF to the TNFR-p55 receptor.

SUMMARY OF THE INVENTION

The subject invention is a molecule that is homologous to tumor necrosis factor (TNF). The invention provides methods and compositions relating to the molecule, HUMAN NTN-2 polypeptide, and related nucleic acids. Included are polypeptides comprising a HUMAN NTN-2-specific domain and having HUMAN NTN-2 -specific activity. The polypeptides may be produced recombinantly from transformed host cells with the subject nucleic acids. The invention provides binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g., genetic hybridization screens for HUMAN NTN-2 transcripts), therapy (e.g., gene therapy to modulate HUMAN NTN-2 gene expression) and in the biopharmaceutical industry (e.g., reagents for screening chemical libraries for lead pharmacological agents).

Preferred uses for the subject HUMAN NTN-2 polypeptides include modifying the physiology of a cell comprising an extracellular surface by contacting the cell or medium surrounding the cell with an exogenous HUMAN NTN-2 polypeptide under conditions whereby the added polypeptide specifically interacts with a component of the medium and/or the extracellular surface to effect a change in the physiology of the cell. Also preferred are methods for screening for biologically active agents, which methods involve incubating a HUMAN NTN-2 polypeptide in the presence of an extracellular HUMAN NTN-2 polypeptide-specific binding target and a candidate agent, under conditions whereby, but for the presence of the agent, the polypeptide specifically binds the binding target at a reference affinity; detecting the binding affinity of the polypeptide to the binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that the agent modulates the binding of the polypeptide to the binding target.

Based upon its homology to TNF, it is expected that HUMAN NTN-2 will be a mediator of immune regulation and inflammatory response, closely linked to the development of disease. It may be useful for regulating development, proliferation and death of cells of the lymphoid, hematopoitic and other lineages. Also, HUMAN NTN-2 may be of use in the prevention of septic shock, autoimmune disorders and graft-host disease. Furthermore, HUMAN NTN-2 polypeptide may be used to identify its receptor.

BRIEF DESCRIPTION OF THE FIGURE

FIGURE 1 - Northern analysis of various human tissue specific RNAs using a 608 nucleotide fragment of the HUMAN NTN-2 sequence as a probe. Lanes 1 - 8 in order as follows: Heart, Brain, Placenta, Lung, Liver, Skeletal Muscle, Kidney and Pancreas.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides HUMAN NTN-2 polypeptide which includes natural HUMAN NTN-2 polypeptide and recombinant polypeptides comprising a HUMAN NTN-2 amino acid sequence, or a functional HUMAN NTN-2 polypeptide domain thereof having an assay-discernable HUMAN NTN-2-specific activity. Accordingly, the polypeptides may be deletion mutants of the disclosed natural HUMAN NTN-2 polypeptides and may be provided as fusion products, e.g., with non- HUMAN NTN-2 polypeptides. The subject HUMAN NTN-2 polypeptide domains have HUMAN NTN-2-specific activity or function.

A number of applications for HUMAN NTN-2 are suggested from its properties. HUMAN NTN-2, may be useful in the study and treatment of conditions similar to those which are treated using TNF. Furthermore, the HUMAN NTN-2 cDNA may be useful as a diagnostic tool, such as through use of antibodies in assays for polypeptides in cell lines or use of oligonucleotides as primers in a PCR test to amplify those with sequence similarities to the oligonucleotide primer, and to see how much HUMAN NTN-2 is present. The isolation of HUMAN NTN-2, of course, also provides the key to isolate its putative receptor, other HUMAN NTN-2 binding polypeptides, and/or study its antagonistic properties.

HUMAN NTN-2-specific activity or function may be determined by convenient in vitro, cellbased, or in vivo assays - e.g., in vitro binding assays, cell culture assays, in animals (e.g., immune response, gene therapy, transgenics, etc.), etc. Binding assays encompass any assay where the specific molecular interaction of a HUMAN NTN-2 polypeptide with a binding target is evaluated. The binding target may be a natural binding target, or a non-natural binding target such as a specific immune polypeptide such as an antibody, or a HUMAN NTN-2 specific agent such as those identified in assays described below.

The claimed polypeptides may be isolated or pure - an "isolated" polypeptide is one that is no longer accompanied by some of the material with which it is associated in its natural state, and that preferably constitutes at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample; a "pure" polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. The subject polypeptides and polypeptide domains may be synthesized, produced by recombinant technology, or purified from cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions, see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY).

The subject polypeptides find a wide variety of uses including use as immunogens, targets in screening assays, bioactive reagents for modulating cell growth, differentiation and/or function, etc. For example, the invention provides methods for modifying the physiology of a cell comprising an extracellular surface by contacting the cell or medium surrounding the cell with an exogenous HUMAN NTN-2 polypeptide under conditions whereby the added polypeptide specifically interacts with a component of the medium and/or the extracellular surface to effect a change in the physiology of the cell. According to these methods, the extracellular surface includes plasma membrane-associated receptors; the exogenous HUMAN NTN-2 refers to a polypeptide not made by the cell or, if so, expressed at non-natural levels, times or physiologic locales; and suitable media include in vitro culture media and physiological fluids such as blood, synovial fluid, etc. The polypeptides may be may be introduced, expressed, or repressed in specific populations of cells by any convenient way such as microinjection, promoter-specific expression of recombinant enzyme, targeted delivery of lipid vesicles, etc.

The invention provides natural and non-natural HUMAN NTN-2-specific binding agents, methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. HUMAN NTN-2-specific binding agents include HUMAN NTN-2-specific receptors, such as somatically recombined protein receptors like specific antibodies or T-cell antigen receptors (See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory) and also includes other natural binding agents identified with assays such as one-, two- and three-hybrid screens, and non-natural binding agents identified in screens of chemical libraries such as described below. Agents of particular interest modulate HUMAN NTN-2 function.

The invention provides HUMAN NTN-2 nucleic acids, which find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc., as well as use in detecting the presence of HUMAN NTN-2 genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional HUMAN NTN-2 homologs and structural analogs.

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e., no longer accompanied by some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. Nucleic acids comprising the nucleotide sequence disclosed herein and fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by a sequence other than that to which it is joined on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, which is immediately flanked by a sequence other than that to which it is joined on a natural chromosome. While the nucleic acids are usually RNA

or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

The amino acid sequences of the disclosed HUMAN NTN-2 polypeptide is used to back translate HUMAN NTN-2 polypeptide-encoding nucleic acids optimized for selected expression systems (Holler, et al. (1993) Gene 136: 323-328; Martin, et al. (1995) Gene 154: 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural HUMAN NTN-2 encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc., Madison, WI). HUMAN NTN-2 encoding nucleic acids may be part of expression vectors and may be incorporated into recombinant host cells, e.g., for expression and screening, for transgenic animals, for functional studies such as the efficacy of candidate drugs for disease associated with HUMAN NTN-2 mediated signal transduction, etc. Expression systems are selected and/or tailored to effect HUMAN NTN-2 polypeptide structural and functional variants through alternative post-translational processing.

The invention also provides for nucleic acid hybridization probes and replication/amplification primers having a HUMAN NTN-2 cDNA specific sequence and sufficient to effect specific hybridization with SEQ. I.D. NO. 1. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M $NaPO_4$, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSPE buffer at 42°C. HUMAN NTN-2 cDNA homologs can also be distinguished from other polypeptides using alignment algorithms, such as BLASTX (Altschul, et al. (1990) Basic Local Alignment Search Tool, J. Mol. Biol. 215: 403-410).

HUMAN NTN-2 hybridization probes find use in identifying wild-type and mutant alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. HUMAN NTN-2 nucleic acids are also used to modulate cellular expression or intracellular concentration or availability of active HUMAN NTN-2. HUMAN NTN-2 inhibitory nucleic acids are typically antisense - single stranded sequences comprising complements of the disclosed natural HUMAN NTN-2 coding sequences. Antisense modulation of the expression of a given HUMAN NTN-2 polypeptide may employ antisense nucleic acids operably linked to gene regulatory sequences. Cells are transfected with a vector comprising a HUMAN NTN-2 sequence with a promoter sequence oriented such that transcription of the gene yields an antisense transcript capable of binding to endogenous HUMAN NTN-2 encoding mRNA. Transcription of the antisense nucleic acid may be constitutive or inducible and the vector may provide for stable extrachromosomal maintenance or integration. Alternatively, single-stranded antisense nucleic acids that bind to genomic DNA or mRNA encoding a given HUMAN NTN-2 polypeptide may be administered to the target cell, in or temporarily isolated from a host, at a concentration that results in a substantial reduction in expression of the targeted polypeptide. An enhancement in HUMAN NTN-2 expression is effected by introducing into the targeted cell type HUMAN NTN-2 nucleic acids which increase the functional expression of the corresponding gene products. Such nucleic acids may be HUMAN NTN-2 expression vectors, vectors which upregulate the functional expression of an endogenous allele, or replacement vectors for targeted correction of mutant alleles. Techniques for introducing the nucleic acids into viable cells are known in the art and include retroviral-based transfection, viral coat protein-liposome mediated transfection, etc.

The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of HUMAN NTN-2 modulatable cellular function. Generally, these screening methods involve

assaying for compounds which modulate HUMAN NTN-2 interaction with a natural HUMAN NTN-2 binding target. A wide variety of assays for binding agents are provided including protein-protein binding assays, immunoassays, cell based assays, etc. Preferred methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds.

In vitro binding assays employ a mixture of components including a HUMAN NTN-2 polypeptide, which may be part of a fusion product with another peptide or polypeptide, e.g., a tag for detection or anchoring, etc. The assay mixtures comprise a natural HUMAN NTN-2 binding target. While native binding targets may be used, it is frequently preferred to use portions thereof as long as the portion provides binding affinity and avidity to the subject HUMAN NTN-2 conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds, preferably small organic compounds, and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents such as salts, buffers, neutral proteins, e.g., albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc., may also be included. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. The mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the HUMAN NTN-2 specifically binds the cellular binding target, portion or analog with a reference binding affinity. Incubation periods are chosen for optimal binding but are also minimized to facilitate rapid, high throughput screening.

After incubation, the agent-biased binding between the HUMAN NTN-2 and one or more binding targets is detected by any convenient way. For cell-

free binding type assays, a separation step is often used to separate bound from unbound components. Separation may be effected by precipitation, immobilization, etc., followed by washing by, e.g., membrane filtration or gel chromatography. For cell-free binding assays, one of the components usually comprises or is coupled to a label. The label may provide for direct detection as radioactivity, luminescence, optical or electron density, etc., or indirect detection such as an epitope tag, an enzyme, etc. A variety of methods may be used to detect the label depending on the nature of the label and other assay components, e.g., through optical or electron density, radiative emissions, nonradiative energy transfers, or indirectly detected with antibody conjugates, etc. A difference in the binding affinity of the HUMAN NTN-2 polypeptide to the target in the absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates the binding of the HUMAN NTN-2 polypeptide to the corresponding binding target. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least a 90% difference.

The invention provides for a method for modifying the physiology of a cell comprising an extracellular surface in contact with a medium, said method comprising the step of contacting said medium with an exogenous HUMAN NTN-2 polypeptide under conditions whereby said polypeptide specifically interacts with at least one of a component of said medium and said extracellular surface to effect a change in the physiology of said cell.

The invention further provides for a method for screening for biologically active agents, said method comprising the steps of a) incubating a HUMAN NTN-2 polypeptide in the presence of an extracellular HUMAN NTN-2 polypeptide specific binding target and a candidate agent, under conditions whereby, but for the presence of said agent, said polypeptide specifically binds said binding target at a reference affinity; b) detecting the binding affinity of said polypeptide to said binding target to determine an agent-

biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that said agent modulates the binding of said polypeptide to said binding target.

One embodiment of the invention is an isolated HUMAN NTN-2 polypeptide comprising the amino acid sequence as set forth herein or a fragment thereof having HUMAN NTN-2-specific activity.

Another embodiment of the invention is a recombinant nucleic acid encoding HUMAN NTN-2 polypeptide comprising the amino acid sequence as set forth herein or a fragment thereof having HUMAN NTN-2-specific activity.

Still another embodiment is an isolated nucleic acid comprising a nucleotide sequence as set forth herein or a fragment thereof having at least 18 consecutive bases and sufficient to specifically hybridize with a nucleic acid having the sequence of set forth herein in the presence of natural HUMAN NTN-2 cDNA.

The present invention also provides for antibodies to the HUMAN NTN-2 polypeptide described herein which are useful for detection of the polypeptide in, for example, diagnostic applications. For preparation of monoclonal antibodies directed toward this HUMAN NTN-2 polypeptide, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

The monoclonal antibodies for diagnostic or therapeutic use may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; Olsson et al., 1982, Meth. Enzymol. 92:3-16). Chimeric antibody molecules may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851, Takeda et al., 1985, Nature 314:452).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of the HUMAN NTN-2 polypeptide described herein. For the production of antibody, various host animals can be immunized by injection with the HUMAN NTN-2 polypeptide, or a fragment or derivative thereof, including but not limited to rabbits, mice and rats. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum.

A molecular clone of an antibody to a selected HUMAN NTN-2 polypeptide epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or antigen binding region thereof.

The present invention provides for antibody molecules as well as fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1 - Cloning and Sequencing of Partial HUMAN NTN-2 Coding Sequence

Amino acid sequences of all the known human and mouse members of the TNF family were used as tblastn queries to search the NIH EST database of random fragments of mRNA sequences (Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. J. Mol. Biol. 215:403-10). Each query generated a list of hits, i.e. EST sequences with a substantial sequence similarity to the query sequence. Typically, the hits on top of the list corresponded to mRNA copies of the query protein, followed by ESTs derived from other members of the family and random-chance similarities.

A parser program was used to combine and sort all the hits from searches with all the members of the family. This allowed rapid subtraction of all the hits corresponding to known proteins. The remaining hits were analyzed for conservation of sequence motifs characteristic for the family. Additional

database searches were performed to identify overlapping ESTs. The partial nucleotide and deduced amino acid sequence of Human NTN-2 was determined as follows:

```
                    10          20          30          40          50
Seq ID#1   ACT GGT TAC TTT TTT ATA TAT GGT CAG GTT TTA TAT ACT GAT AAG ACC TAC GCC ATG
Seq ID#2   Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met>

            60          70          80          90          100         110
           GGA CAT CTA ATT CAG AGG NAG AAG GTC CAT GTC TTT GGG GAT GAA TTG AGT CTG GTG
           Gly His Leu Ile Gln Arg Xxx Lys Val His Val Phe Gly Asp Glu Leu Ser Leu Val>

               120         130         140         150         160         170
           ACT TTG TTT CGA TGT ATT CAA AAT ATG CCT GAA ACA CTA CCC AAT AAT TCC TGC TAT
           Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr>

                   180         190         200         210         220
           TCA GCT GGC ATT GCA AAA CTG GAA GAA GGA GAT GAA CTC CAA CTT GCA ATA CCA AGA
           Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg>

          230         240         250         260         270         280
           GAA AAT GCA CAA ATA TCA CTG GAT GGA GAT GTC ACA TTT TTT GGT GCA TTG AAA CTG
           Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu>

              290
           CTG TGA
           Leu ***>
```

Using the nucleotide sequence of SEQ. I.D. NO. 1 as a query, additional database searches were performed to identify overlapping ESTs. Two additional clones from the I.M.A.G.E. consortium were discerned to contain homologous sequence. These clones, GeneBank Accession Nos. AA166695 and T87299 were obtained from Research Genetics, Inc. (Huntsville, AL) and sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). Alignment of the two additional clones with SEQ. I.D. NO. 1 indicated a total length of 680 nucleotides. Oligonucleotides were designed based on the partial human sequence and used as primers for the reverse transcriptase reaction and for PCR. A 608 nucleotide long sequence was obtained and used as a probe to isolate the full length sequence as described below.

EXAMPLE 2 - ISOLATION AND SEQUENCING OF FULL LENGTH cDNA CLONE ENCODING HUMAN NTN-2

A human placenta cDNA library in lambda gt-10 was obtained from Clontech Laboratories, Inc. (Palo Alto, CA). Plaques were plated at a density of 1.25 x 10^6/20x20 cm plate, and replica filters taken following standard procedures (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., page 8.46, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). Filters were screened at normal stringency (2 x SSC, 65°C) with a probe corresponding to nucleotides 216 to 824 of the hNTN-2 sequence shown in SEQ. I.D. NO. 3. The probe was hybridized at 65°C in hybridization solution containing 0.5 mg/ml salmon sperm DNA to decrease non-specific binding of the probe to the filter. Filters were washed in 2 x SSC at 65° C and exposed overnight to X-ray film. Five positive clones were picked that showed strong hybridization signals and also produced fragments when PCR-amplified using oligos from the cDNA vector.

Sequencing of hNTN-2

The coding region from each of the five clones was sequenced using the ABI 373A DNA sequencer and Taq Dyedeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). The nucleotide and deduced amino acid sequence of the full length hNTN-2 coding sequence obtained from one of the clones is set forth as follows:

```
                10          20          30          40          50          60
                .           .           .           .           .           .
SEQ ID #3  ATG GAT GAC TCC ACA GAA AGG GAG CAG TCA CGC CTT ACT TCT TGC CTT AAG AAA AGA GAA
SEQ ID #4  Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu Lys Lys Arg Glu>

                70          80          90          100         110         120
                .           .           .           .           .           .
GAA ATG AAA CTG AAG GAG TGT GTT TCC ATC CTC CCA CGG AAG GAA AGC CCC TCT GTC CGA
Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro Arg Lys Glu Ser Pro Ser Val Arg>

                130         140         150         160         170         180
                .           .           .           .           .           .
TCC TCC AAA GAC GGA AAG CTG CTG GCT GCA ACC TTG CTG CTG GCA CTG CTG TCT TGC TGC
Ser Ser Lys Asp Gly Lys Leu Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys>

                190         200         210         220         230         240
                .           .           .           .           .           .
CTC ACG GTG GTG TCT TTC TAC CAG GTG GCC GCC CTG CAA GGG GAC CTG GCC AGC CTC CGG
Leu Thr Val Val Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg>

                250         260         270         280         290         300
                .           .           .           .           .           .
GCA GAG CTG CAG GGC CAC CAC GCG GAG AAG CTG CCA GCA GGA GCA GGA GCC CCC AAG GCC
Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly Ala Pro Lys Ala>

                310         320         330         340         350         360
                .           .           .           .           .           .
GGC CTG GAG GAA GCT CCA GCT GTC ACC GCG GGA CTG AAA ATC TTT GAA CCA CCA GCT CCA
Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro>

                370         380         390         400         410         420
                .           .           .           .           .           .
GGA GAA GGC AAC TCC AGT CAG AAC AGC AGA AAT AAG CGT GCC GTT CAG GGT CCA GAA GAA
Gly Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu>

                430         440         450         460         470         480
                .           .           .           .           .           .
ACA GTC ACT CAA GAC TGC TTG CAA CTG ATT GCA GAC AGT GAA ACA CCA ACT ATA CAA AAA
Thr Val Thr Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys>

                490         500         510         520         530         540
                .           .           .           .           .           .
GGA TCT TAC ACA TTT GTT CCA TGG CTT CTC AGC TTT AAA AGG GGA AGT GCC CTA GAA GAA
Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu>

                550         560         570         580         590         600
                .           .           .           .           .           .
AAA GAG AAT AAA ATA TTG GTC AAA GAA ACT GGT TAC TTT TTT ATA TAT GGT CAG GTT TTA
Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu>

                610         620         630         640         650         660
                .           .           .           .           .           .
TAT ACT GAT AAG ACC TAC GCC ATG GGA CAT CTA ATT CAG AGG AAG AAG GTC CAT GTC TTT
Tyr Thr Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe>

                670         680         690         700         710         720
                .           .           .           .           .           .
GGG GAT GAA TTG AGT CTG GTG ACT TTG TTT CGA TGT ATT CAA AAT ATG CCT GAA ACA CTA
Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu>

                730         740         750         760         770         780
                .           .           .           .           .           .
CCC AAT AAT TCC TGC TAT TCA GCT GGC ATT GCA AAA CTG GAA GAA GGA GAT GAA CTC CAA
Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln>

                790         800         810         820         830         840
                .           .           .           .           .           .
CTT GCA ATA CCA AGA GAA AAT GCA CAA ATA TCA CTG GAT GGA GAT GTC ACA TTT TTT GGT
Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly>

                850
                .
GCA TTG AAA CTG CTG TGA
Ala Leu Lys Leu Leu ***>
```

EXAMPLE 3 - TISSUE SPECIFIC EXPRESSION OF hNTN-2

A fragment corresponding to nucleotides 216 to 824 of the hNTN-2 sequence shown in SEQ. I.D. NO. 3 was radiolabeled and utilized in Northern analysis of various human tissue specific RNAs. The Northern blot containing polyA+ RNA from several human tissues was obtained from Clontech Laboratories, Inc. (Palo Alto, CA) and was hybridized at 65°C to the radiolabeled hNTN-2 probe in the presence of 0.5M NaPO4 (pH 7), 1% bovine serum albumin (Fraction V, Sigma), 7% SDS, 1 mM EDTA and 100 ng/ml sonicated, denatured salmon sperm DNA. The filter was washed at 65°C with 2X SSC, 0.1% SDS and subjected to autoradiography for 16 hours with one intensifying screen and X-ray film at -70°C.

The hNTN-2 probe hybridized strongly to a 2.7 kb transcript in human heart, placenta, pancreas and lung tissue (Figure 1) and hybridized weakly to RNA from brain and liver. Weaker levels of expression could also be found in skeletal muscle and kidney. High expression of hNTN-2 in heart tissue may suggest that the present invention may be utilized to treat heart disease. Expression of hNTN-2 in lung and pancreas tissue may suggest that the present invention may be utilized to treat lung and/or pancreas related disorders.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding HUMAN NTN-2.

2. An isolated nucleic acid molecule according to claim 1, having a sequence selected from the group consisting of:
   (a) the nucleotide sequence comprising the coding region of the HUMAN NTN-2 as set forth in SEQ. I.D. NO. 3;
   (b) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of (a) and which encodes a molecule having the biological activity of the HUMAN NTN-2; or
   (c) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a nucleotide sequence of (a) or (b), and which encodes a molecule having the biological activity of the HUMAN NTN-2.

3. A vector which comprises a nucleic acid molecule of claim 1 or 2.

4. A vector according to claim 3, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.

5. A vector according to claim 3 or 4, which is a plasmid.

6. Isolated HUMAN NTN-2 polypeptide encoded by the nucleic acid molecule of claim 1 or 2.

7. Isolated HUMAN NTN-2 polypeptide, having the amino acid sequence as set forth in SEQ. I.D. NO. 4.

8. A host-vector system for the production of HUMAN NTN-2 which comprises a vector of claim 3 or 4, in a host cell.

9. A host-vector system according to claim 8, wherein the host cell is a bacterial, yeast, insect or mammalian cell.

10. A method of producing HUMAN NTN-2 which comprises growing cells of a host-vector system of claim 8 or 9, under conditions permitting production of the cerberus, and recovering the HUMAN NTN-2 so produced.

11. An antibody which specifically binds the HUMAN NTN-2 of claim 6 or 7.

12. An antibody according to claim 11, which is a monoclonal antibody.

13. A pharmaceutical composition comprising HUMAN NTN-2 according to claim 6 or 7, and a pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising an antibody according to claim 11 or 12, and a pharmaceutically acceptable carrier.

15. HUMAN NTN-2 according to claim 6 or 7, an antibody according to claim 11 or 12, or a composition according to claim 13 or 14, for use in a method of treatment of the or animal body, or in a method of diagnosis.

16. A polypeptide produced by the method of claim 10.

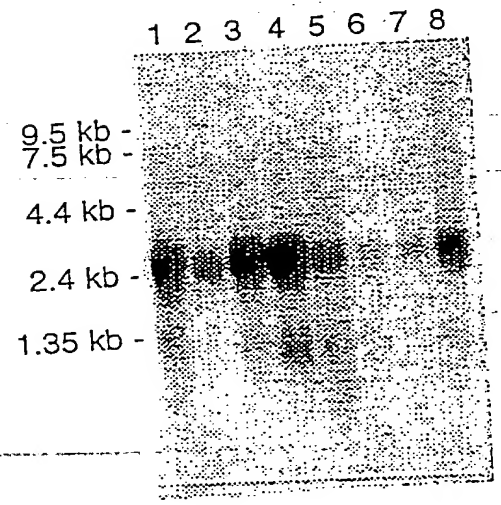
17. A ligandbody which comprises HUMAN NTN-2 fused to an immunoglobulin constant region.

18. The ligandbody of claim 17, wherein the immunoglobulin constant region is the Fc portion of human IgG1.

19. A ligandbody according to claim 17 or 18, for use in a method of treatment of the human or animal body, or in a method of diagnosis.

20. A polypeptide comprising the amino acid sequence as set forth in SEQ. I.D: NO. 4.

Fig. 1



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/11294

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/19 C07K14/52 C07K16/24 C07K19/00 A61K38/19
C12Q1/68 A61K39/395

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	L. HILLIER ET AL: "WashU-NCI human EST project, zo85e02.s1 Stratagene ovarian cancer(#9372219) Homo sapiens cDNA clone 593690 3'" EMBL DATABASE ENTRY HSAA66695, ACCESSION NUMBER AA1166695,21 December 1996, XP002072308 cited in the application see abstract	1,2
A	L. HILLIER ET AL: "The WashU-Merck EST project, yd89b02.s1 Homo sapiens cDNA clone 115371 3'" EMBL DATABASE ENTRY HS29950, ACCESSION NUMBER T87299,31 March 1995, XP002078459 cited in the application see abstract	1,2

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
23 September 1998	09/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Cornec, N

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intel ...nal Application No
PCT/US 98/11294

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 18921 A (HUMAN GENOME SCIENCES, INC.) 7 May 1998 * see the whole document especially sequences ID 1 and 2, claims, examples, pages 50-55 *	1-13,15, 16,20
E	WO 98 27114 A (SCHERING CORPORATION) 25 June 1998 * see the whole document especially sequences ID 3 and 4*	1-13,15, 16,20

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ional Application No
PCT/US 98/11294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9818921 A	07-05-1998	AU 7674596 A	22-05-1998
WO 9827114 A	25-06-1998	AU 5705898 A	15-07-1998